Biliary amphotericin B pharmacokinetics and pharmacodynamics in critically ill liver transplant recipients receiving treatment with amphotericin B lipid formulations

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Abstract: Fungal cholangitis is a potentially life-threatening condition. As amphotericin B (AmB) has a broad antimycotic spectrum, in this study its biliary penetration and activity was determined in two patients treated with liposomal AmB (L-AmB) and in one patient receiving AmB colloidal dispersion (ABCD). Biliary and plasma AmB levels were quantified by high-performance liquid chromatography after purification by solid-phase extraction. For assessment of biliary AmB activity, isolates of Candida albicans, Candida tropicalis, Candida glabrata and Candida krusei were incubated in porcine bile at AmB concentrations of 0.025-5.00 mg/L. In addition, patient bile samples retrieved for AmB quantification were inoculated with the same Candida strains. Biliary AmB concentrations were lower and displayed a slower rise and decline than plasma levels. The highest penetration ratio, as expressed by the ratio between the area under the AmB concentration-time curve in bile and plasma (liberated AmB) over the sampling period (AUC0-n bile/AUC0-n LI plasma), was 0.28. Proliferation of C. albicans and C. tropicalis in bile was similar to that in culture medium, whereas growth of C. glabrata was diminished and proliferation of C. krusei was absent in bile. In comparison with culture medium, AmB activity decreased in spiked porcine bile. In all but one patient bile sample, fungal growth was delayed or lacking even when AmB was not detectable. However, no fungicidal effect was observed in patient bile at AmB concentrations up to 1.28 mg/L. Thus, a reliable response of fungal cholangitis to treatment with L-AmB or ABCD cannot be anticipated.

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Biliary Amphotericin B Pharmacokinetics and Pharmacodynamics in Critically Ill Liver Transplant Recipients on Treatment with Amphotericin B Lipid Formulations

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ABSTRACT

Fungal cholangitis is a potentially life-threatening condition. As amphotericin B (AMB) has a broad antimycotic spectrum, its biliary penetration and activity was determined in two patients treated with liposomal AMB (LAMB) and in one patient on AMB colloidal dispersion (ABCD). Biliary and plasma AMB were quantified by high-pressure-liquid-chromatography after purification by solid phase extraction. For assessment of biliary AMB activity, isolates of *Candida (C.) albicans, C. tropicalis, C. glabrata* and *C. krusei* were incubated in porcine bile at AMB concentrations of 0.025 - 5.00 mg/L. Additionally, patient bile samples retrieved for AMB quantification were inoculated with the same *Candida* strains. Biliary AMB concentrations were lower and displayed a slower rise and decline than plasma levels. The highest penetration ratio as expressed by the ratio between the area under the time-AMB-concentration curve in bile and plasma (liberated AMB) over the sampling period ($AUC_{0\text{-n bile}}/AUC_{0\text{-n LI plasma}}$) amounted to 0.28. Proliferation of *C. albicans* and *C. tropicalis* in bile was similar to that in culture medium whereas growth of *C. glabrata* was diminished and proliferation of *C. krusei* was absent in bile. In comparison with medium, AMB activity decreased in spiked porcine bile. In all but one patient bile sample, fungal growth was delayed or lacking, even when AMB was not detectable. However, no fungicidal effect was observed in patient bile at AMB concentrations of up to 1.28 mg/L. Thus, a reliable response of fungal cholangitis to treatment with LAMB or ABCD cannot be anticipated.

1. Introduction

Fungal cholangitis is a life-threatening condition affecting mainly immune-compromised persons, patients with choledocholithiasis, cancer, bile duct strictures, primary sclerosing cholangitis or liver transplant recipients [1-3]. Liver abscess, severe sepsis and septic shock are frequent complications. Data on biliary concentrations of antifungals are scarce. Because of its broad fungicidal activity, amphotericin B (AMB) is still a cornerstone in the treatment of invasive fungal infections (IFIs). AMB has a high protein binding exceeding 90 percent. After administration of its conventional deoxycholate formulation, it is eliminated via urine and bile. As AMB deoxycholate displays a considerable infusion-related and renal toxicity, less toxic lipid formulations such as liposomal AMB (LAMB) or AMB colloidal dispersion (ABCD) are preferred in intensive care medicine. Therefore, we assessed biliary and plasma AMB levels during treatment with LAMB or ABCD in critically ill patients.

2. Patients and methods

2.1. Pharmacokinetic analysis

Adult critically ill patients on treatment with lipid-formulated AMB for proven or suspected IFI and an indication for retrieval of bile were enrolled. LAMB (AmBisome®, Gilead, Foster City, CA, USA) and ABCD (Amphocil®, Chiesi Pharmaceuticals, Vienna, Austria) were infused at standard doses (~ 3-5 mg/kg once daily) over four hours for suspected or proven IFI. The first ABCD dose amounted to 50 percent of the maintenance dose in order to avoid infusion-related toxicity. The bile collection bags were changed before AMB infusion as well as 4, 6, 10, 16 and 24 hours after start of infusion. Heparinised 2-mL blood samples were drawn whenever the collection bag was changed. Bile and blood sampling was performed on day 1 of therapy with lipid-formulated AMB and at approximately steady state (day 4 in

Patient 3, day 5 in patient 2 and day 7 in Patient 1). Blood samples were centrifuged immediately. Bile and plasma were stored at

-80 °C. In plasma, lipid-bound AMB and AMB that had been liberated from lipid-encapsulation were separately quantified as described previously [4]. For bile samples, the method had to be modified because of high viscosity of bile and suboptimal signal noise ratio. Bile was filtrated (Filtropur S 0.2 µm pore size; Sarstedt, Nümbrecht, Germany). Subsequently, 500 µL of filtered bile was treated with 1 mL of dimethyl sulfoxide (Merck, Darmstadt, Germany) and methanol (Rotisolv®, Carl Roth, Karlsruhe, Germany; 1:1, v/v). For high performance liquid chromatography, a Zorbax 300SB-C18 column (Agilent Technologies, Vienna, Austria) was used. The mobile phase consisted of acetonitrile (Rotisolv®, Carl Roth, Karlsruhe, Germany) and 10 mM NaH_2PO_4 (Merck, Darmstadt, Germany; 45:55, v/v). Inter and intra-day variability for biliary total AMB levels were < 15 percent, the lower limit of quantification (LLOQ) < 0.01 mg/L. Liberated and lipid-bound AMB could be separated in one bile specimen obtained by endoscopy (data not shown). In all bile samples taken from Patient 1-3, only total AMB could be measured. However, we assume that total biliary AMB comprises exclusively liberated AMB since no lipid-bound AMB penetrates into bile. Therefore, "biliary AMB" means total biliary AMB which consists of liberated AMB (see 4. Discussion). Based on this assumption, the penetration ratio for AMB into bile was defined as the ratio between the area under the time-total-AMB-concentration curve in bile and the area under the time-liberated-AMB-concentration curve in plasma over the sampling period ($AUC_{0\text{-}n\ bile}/AUC_{0\text{-}n\ LI\ plasma}$) [5]. Total AMB plasma levels were obtained by addition of plasma concentrations of liberated and lipid-bound AMB. Pharmacokinetics were calculated by a non-compartmental model using Kinetica-2000® (InnaPhase Corporation, Champs-sur-Marne, France). The area under the concentration-time curve over the sampling period ($AUC_{0\text{-}n}$) was computed using the log linear method, whenever the

concentration in a trapezoid decreased, or with the trapezoidal method when the concentration increased.

2.2. Microbiological diagnostics

Candida (C.) krusei and *C. glabrata* were cultured from bile of Patient 3. Minimum inhibitory concentration (MIC) values were determined for isolates obtained from Patient 3. Susceptibility testing was performed by the agar based ETest method (AB Biodisk, Solna, Sweden) using RPMI-2G agar plates (Sigma, Vienna, Austria). According to the manufacturer's recommendations (Etest technical guide number 4. Antifungal susceptibility of yeasts, AB Biodisk, Solna, Sweden) plates were inoculated by dipping a sterile swab into the inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard and streaked across the agar surface in three directions. Agar plates were dried for at least 15 minutes before applying the ETest strips. AMB MICs were determined after 24 to 48 h of incubation at 37 °C as the lowest drug concentration inhibiting any visible growth (100 percent).

2.3. In-vitro and ex-vivo simulations

Candida isolates (*C. albicans, C. tropicalis, C. glabrata* and *C. krusei*), all deep-frozen for storage, were grown on Sabouraud glucose (SAB) agar plates for 24 h at 36 ± 1 °C. Patient bile samples and porcine bile were filtered in order to abolish any bacterial or fungal contamination (Filtropur S 0.45 µm and 0.2 µm pore size, Sarstedt, Nümbrecht, Germany). Subsequently, samples were inoculated with 1 x 10^4 *Candida* cells. Controls were performed in culture medium (RPMI 1640 medium, Sigma-Aldrich, Austria) at pH 7.2 and in RPMI adjusted to biliary pH which was 7.8 with NaOH. Fungal suspensions (40 µl) were added to patient bile samples, porcine bile, RPMI at pH 7.2 and RPMI at pH 7.8 (sample volume 3 ml). Tubes were gently shaken for 48 h at 36 ± 1 °C. After 7, 12, 24 and 48 hours, respectively, aliquots of 100 µl were drawn and diluted 10-fold or 100-fold in double-distilled water. Aliquots (50 µl) of these dilutions were plated in duplicate on SAB agar plates with an automatic spiral plater (model WASP 2, Don Whitley Scientific, Shipley, United Kingdom).

The plates were incubated at 37 ± 1 °C for 24 h and the numbers of fungal CFU were counted and assessed, considering the duplicity and the dilution [6].

Fungal growth in bile and effects of bile on AMB pharmacodynamics were investigated *in-vitro*. Filtered porcine bile was spiked with 0.0 (control), 0.025, 0.05, 0.5, 1.0 and 5.0 mg/L of AMB deoxycholate (conventional AMB) and inoculated with *C. albicans, C. tropicalis, C. glabrata* and *C. krusei* (10^4 conidia per inoculum).

For assessment of antifungal activity of AMB in patient bile samples *ex-vivo* simulation was performed. Patient bile samples retrieved for AMB quantification were filtered and inoculated with the same *Candida* strains (10^4 conidia per inoculum). For comparison, filtered porcine bile and medium at pH 7.2 and pH 7.8 were spiked with AMB deoxycholate at concentrations measured in patient bile samples.

3. Results

3.1. Patients

Three patients were enrolled (see Table 1). Patient 1 and Patient 2 had recently undergone orthotopic liver transplantation and bile sampling was performed via T-tubes which had been inserted at the operation. Patient 3 was also a liver transplant recipient and presented with liver abscesses due to strictures of his bile duct anastomosis. Therefore, bile was deviated via bile duct drainage. *C. krusei* and *C. glabrata* were cultivated from bile of Patient 3. MIC values are displayed in Table 1.

3.2. Biliary and plasma pharmacokinetics of amphotericin B

As displayed in Figure 1, biliary AMB concentrations were lower than the simultaneous plasma levels of total, lipid-bound and liberated AMB. The rise of AMB concentrations in bile and their decline were delayed in comparison with plasma. Patient 1 presented the highest

biliary AMB concentrations (maximum biliary AMB 1.28 mg/L) on day 7 of LAMB therapy. Whereas AMB was already detectable in bile collected after the first LAMB infusion (Patient 1), no AMB could be recovered from bile within 24 hours after a single 200-mg dose of ABCD (Patient 3). In Patient 1, penetration ratios amounted to 0.15 and 0.28 after single and multiple doses, respectively. In Patient 2 and Patient 3 penetration ratios were 0.12 and 0.05, respectively, after multiple doses. Plasma pharmacokinetics of liberated, lipid-bound, and total AMB during treatment with LAMB (Patient 1 and Patient 2) or ABCD (Patient 3) are summarized in Table 2.

3.3. Biliary amphotericin B pharmacodynamics assessed by in-vitro simulations

Growth of *C. albicans* and *C. tropicalis* in native porcine bile (pH 7.8) was comparable to that in RPMI medium at pH 7.2 and at pH 7.8. The antifungal effect of AMB, however, was different in porcine bile and in medium. In medium containing AMB at a concentration of 5 mg/L, *C. albicans* and *C. tropicalis* were eradicated after an exposure of 7 to 24 hours. At a concentration of 1 mg/L, the number of CFUs of *C. albicans* remained almost constant over a 24 h-incubation period but CFU count of *C. tropicalis* slightly declined. In bile, however, an AMB concentration of 5 mg/L stopped proliferation, but did not result in a reduction of CFU of *C. albicans* or *C. tropicalis*. Lower concentrations were completely ineffective.

By contrast, proliferation of *C. glabrata* in porcine bile was lower than in medium and was inhibited by biliary AMB concentrations at 1 mg/L and 5 mg/L. Unlike in bile, in culture medium, *C. glabrata* was eradicated by 1 mg/L and 5 mg/L of AMB at pH 7.8 and by 5 mg/L of AMB at pH 7.2. *C. krusei* did not display any proliferation in porcine bile and CFU remained unchanged over a 48 h-incubation in native and in AMB-spiked porcine bile. Thus, in bile, none of the tested strains was killed even when exposed to an AMB-concentration of 5 mg/L (Table 3).

3.4. Biliary amphotericin B pharmacodynamics assessed by ex-vivo simulations

Data obtained from *ex-vivo* simulations are summarized in Table 4. In bile samples obtained from Patient 1 treated with LAMB, CFU of *C. tropicalis* and *C. albicans* remained constant. AMB at concentrations of up to 1.28 mg/L had no fungicidal effect. A slow and inconstant growth of *C. albicans* was observed in bile samples of Patient 2 (also on LAMB) at AMB concentrations of 0.40 mg/L or below. In bile samples of Patient 3 who received ABCD, no AMB was detectable after the first dose and AMB concentrations were still very low on day 4 of treatment. Inoculation with *C. krusei* or *C. glabrata* resulted in variable growth that was slower than in medium. Thus, AMB concentrations of 0.04 mg/L or below had no obvious effect on proliferation of *C. krusei* and *C. glabrata.*

4. Discussion

During treatment with LAMB or ABCD, biliary AMB levels were lower than in plasma. AMB plasma pharmacokinetics were comparable with previously reported data [7, 8]. When administered as a lipid formulation such as LAMB or ABCD, AMB is slowly released from its lipid encapsulation in the plasma. This liberated AMB fraction comprises unbound (ultrafiltrable) and protein-bound AMB. As plasma protein binding of AMB amounts to > 90 percent depending on its concentration most of the liberated AMB is bound to plasma protein. In addition, there is a fraction of lipid-bound AMB [9, 10]. In the present study, liberated and lipid-bound AMB were separately quantified in the plasma but separation of the unbound and the protein-bound fraction has not been performed. Thus, the term “liberated AMB” comprises unbound and protein-bound AMB. “Total AMB” comprises liberated and lipid-bound AMB [4]. Plasma pharmacokinetics of lipid-bound AMB depends on the applied formulation. [10]. In bile samples, only total AMB has been determined. Based on

measurements in a single bile sample (data not shown) we suppose that biliary AMB consists exclusively of liberated AMB. Therefore, we compared biliary AMB concentrations which liberated AMB plasma levels. It remains to be clarified to what extent biliary AMB is bound to proteins or other biliary components.

Published data on AMB penetration into bile are limited. In a dog model, biliary AMB concentration amounted to 0.75 mg/L one day after administration of AMB deoxycholate. Biliary obstruction led to slightly increased AMB serum levels [11]. In studies on isolated perfused rat liver, 1-3 percent of administered AMB deoxycholate but only 0.01-0.08 percent of LAMB could be recovered from bile within 2 hours [12, 13]. A concentration of 5 mg/L exceeding the simultaneous plasma levels was reached by AMB deoxycholate therapy in bile of a young patient suffering from *Candida* cholecystitis [14]. Biliary AMB levels exceeding 40 mg/L were measured in a cancer patient treated with AMB deoxycholate and subsequently with AMB lipid complex (Abelcet®) which is a third clinically used lipid formulation [15]. Faecal AMB excretion by healthy volunteers was much lower after infusion of LAMB than after AMB deoxycholate (4 vs. 43 percent of the administered dose within a week) [16]. In our study cohort, biliary AMB concentrations were lowest in Patient 3 who displayed the highest plasma bilirubin level. However, systemic clearance of liberated AMB was only slightly delayed in patients with cholestatic liver disease treated with ABCD suggesting a limited role of biliary excretion [8]. From different body fluids such as ascites, pleural effusion and epithelial lining fluid, only liberated AMB at relatively low concentrations was recovered [17-19]. In a single bile sample, we succeeded in separation of lipid-bound from liberated AMB but we detected only liberated AMB (data not shown). Thus, only small amounts of AMB, probably the liberated component, appear to be excreted via the bile during treatment with LAMB or ABCD. By contrast, in liver tissue, AMB concentrations of ~ 100 µg/g were measured, probably because of accumulation in reticuloendothelial cells [20].

For comparison, a biliary fluconazole peak level of 11.6 mg/L was measured after a single 200 mg-infusion in a patient with a *Candida* cholecystitis [21]. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) the MIC break point of fluconazole susceptibility is ≤ 2 mg/L for *C. albicans*, *C. krusei* and *C. tropicalis* and ≤ 0.002 mg/L for *C. glabrata*. Caspofungin reached a biliary concentration of 1.0 mg/L after a 70 mg-infusion, and micafungin 1.9 mg/L after administration of 150 mg [22, 23]. The EUCAST break point for susceptibility is ≤ 0.016 mg/L for *C. albicans* and ≤ 0.03 mg/L for *C. glabrata*. The two non-*albicans Candida* strains isolated from bile of Patient 3 displayed MIC values – as determined by ETest - substantially exceeding the biliary AMB concentrations achieved by ABCD treatment. The EUCAST MIC breakpoint of AMB susceptibility is ≤ 1 mg/L for most *Candida* species. AMB MIC values of 0.03 - 4 mg/L have been recently identified for *Candida* isolates with a value of 1 mg/L in numerous isolates, particularly of *Candida non-albicans* strains [24-26]. Only in Patient 1, LAMB yielded biliary AMB concentrations slightly above 1 mg/L at day 7 of treatment. In the bile samples of Patient 2 and Patient 3, AMB concentrations achieved by treatment with LAMB or ABCD were markedly lower. High peak levels, however, would be crucial for antifungal efficacy as AMB displays concentration-dependent pharmacodynamics [27]. The clinical impact of target-site concentrations in relation to MICs determined by E-Test *in-vitro* is not yet clear. The chemical properties of the respective target compartment may influence pharmacodynamics of antimicrobial agents. Therefore, we assessed proliferation of typical fungal pathogens in bile *in-vitro* and *ex-vivo* in order to detect eventual effects of this particular matrix on fungal growth as well as on antimycotic activity of AMB. The effect of porcine bile on proliferation of *Candida* turned out to be variable. However, delayed fungal growth, even in the absence of AMB, was observed in most of the bile samples, particularly in *ex-vivo* samples. This suggests a potential inhibitory effect of certain biliary components on *Candida*. A decrease of

C. albicans metabolism in presence of bile acids at concentrations of 20-240 mg/L has been demonstrated by microcalorimetry. Cholic acid had the strongest effect, followed by glycocholic acid and taurocholic acid [28]. Obviously, this effect does not generally protect against fungal cholangitis or cholecystitis, particularly in patients at high risk.

Unlike in culture medium, fungal killing could not be achieved by incubation with AMB, in any of our human and porcine bile samples, even at a concentration of 5 mg/L which exceeds the biliary AMB levels reached in our study patients. Therefore, an inhibitory effect of bile on antifungal activity of AMB has to be considered. Reduced AMB susceptibility of *C. albicans* in the presence of porcine or bovine bile extract (6.25 percent w/v) with an increase in MIC (32 mg/L versus 1 mg/L) has been previously reported [29]. Similarly, addition of only 0.8 percent of bovine bile to culture medium led to a decrease in AMB susceptibility of *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. The susceptibility of *C. albicans* to AMB, however, was maintained in presence of 0.8 percent of bile [30].

Interpretation of our pharmacokinetic data requires consideration of the specific conditions of our study cohort comprising only three patients. All patients were liver transplant recipients with cholestatic graft dysfunction. Thus, in patients with normal liver function, biliary AMB excretion might be markedly higher. Furthermore, subnormal albumin levels may have influenced plasma protein binding of liberated AMB. An undefined part of excreted bile was collected via T-tube or bile duct drainage. Hence, calculation of the biliary total AMB excretion was not feasible. Unlike in plasma, liberated and lipid-bound AMB could be separated in one bile sample only. Patient 1 and Patient 3 suffered from acute renal failure requiring renal replacement by continuous veno-venous haemofiltration (CVVH). However, CVVH has probably no significant influence on pharmacokinetics of the liberated AMB fraction during treatment with LAMB or ABCD [7]. *In-vitro* MICs were determined in three isolates from one patient only. Concerning *in-vitro* simulations, differences in composition of

human and porcine bile have to be taken into account. *Ex-vivo* simulation revealed impaired growth of the investigated *Candida* strains in human bile. This raises questions on their pathogenetic role in cholangitis. The small number of arbitrarily selected – albeit clinically relevant - *Candida* strains tested *in-vitro* and *ex-vivo* is a limitation of our study.

In conclusion, biliary total AMB concentrations achieved in critically ill liver transplant recipients by treatment with LAMB or ABCD were close to or even markedly below the *in-vitro* MIC values of relevant pathogens. Biliary AMB is probably exclusively liberated AMB. In addition, the results of *in-vitro* and *ex-vivo* simulations suggest an inhibitory effect of bile on the antifungal activity of AMB. Based on these findings and given the lack of clinical outcome data, the efficacy of LAMB and ABCD for treatment of fungal cholangitis appears to be questionable.

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Declarations

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Sharp and Dohme, Pfizer, Schering Plough and Astellas Pharma. All other authors: none to declare with regard to this publication.

Ethical approval: The study was performed according to Good Clinical Practice guidelines and the Declaration of Helsinki. It was approved by the local ethics committee. Written, informed consent was granted by competent patients, post-hoc consent by patients who were incompetent at enrolment.

Previous presentation of data: part of the data of this study has been presented at the 20th Scientific Symposium of the Austrian Pharmacological Society APHAR, 26 – 27 September 2014, Innsbruck, Austria and at the 33rd Vienna Intensive Care Days, 11 – 14 February, 2015, Vienna, Austria

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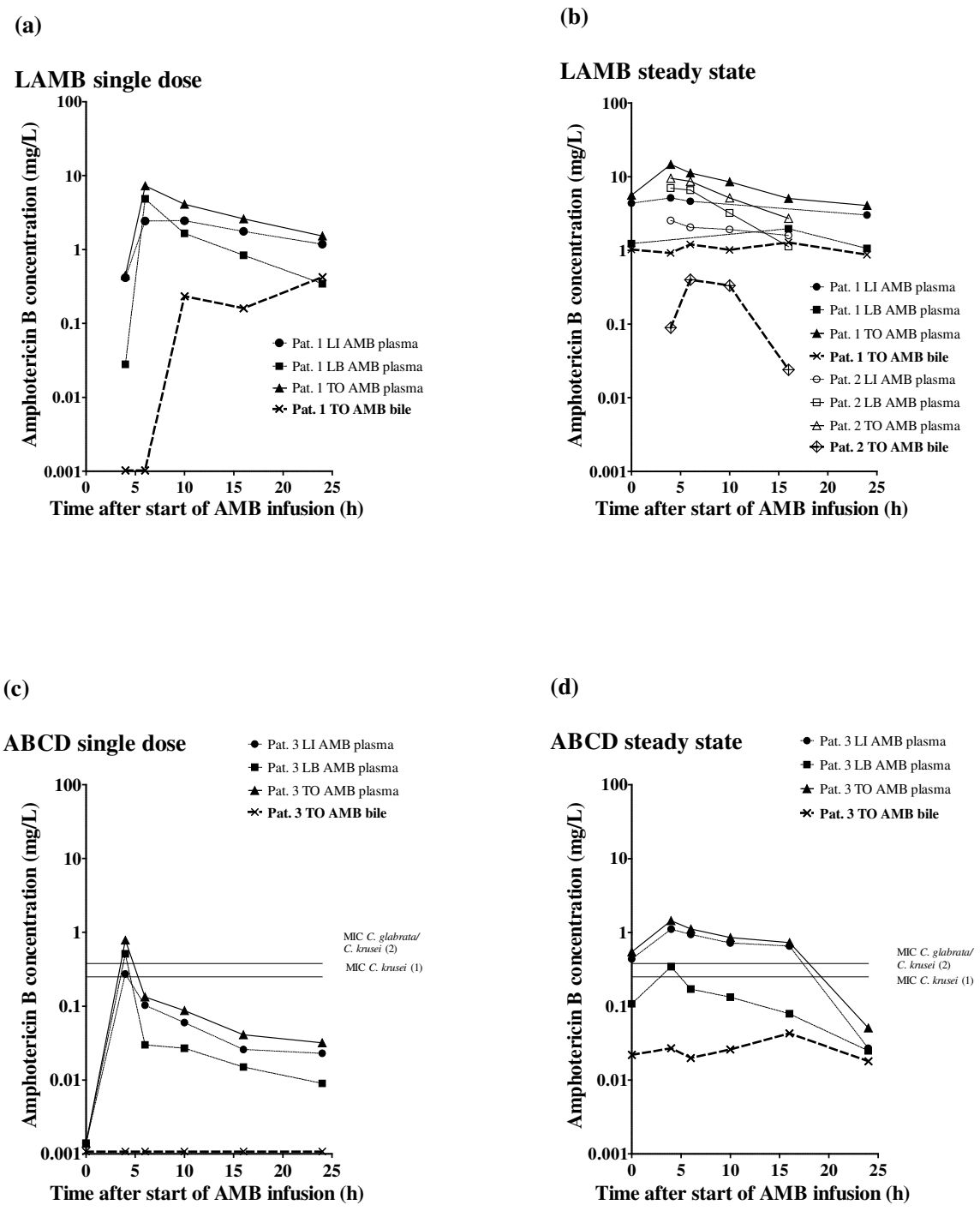

Figure 1: Amphotericin B (AMB) concentration-time profiles in plasma and in bile during treatment with liposomal AMB (LAMB, Panel A and B) and AMB colloidal dispersion (ABCD, Panel C and D)

Legend to Figure 1

Amphotericin B (AMB) concentration-time profiles in plasma and in bile during treatment with liposomal amphotericin B (LAMB), (Panels (a) and (b)), and amphotericin B colloidal dispersion (ABCD), (Panels (c) and (d)); LI, AMB that has been liberated from its lipid-encapsulation; LB, lipid-bound AMB; TO, total AMB (calculated by addition of liberated and lipid-bound AMB). The respective blood samples for measurement of plasma concentrations were drawn at the end of each bile collection, whenever the collection bag was changed (before as well as 4, 6, 10, 16 and 24 h after start of infusion). In plasma, liberated AMB and lipid-bound AMB were separately quantified. In the bile samples, separation was not performed and total AMB was determined. Total biliary AMB, however, probably comprises exclusively liberated AMB; (1) first isolate of *C. krusei*; (2) second isolation of *C. krusei* 36 days later; *C.*, *Candida*

Table 1: Demographic and clinical characteristics of the patients

Patient	**1**	**2**	**3**
Age (years)	60	57	67
Sex	male	female	male
Body weight (kg)	90	53	72
CVVH	yes	no	yes
Laboratory values			
Creatinine (mg/dL)	0.69	1.51	2.89
Bilirubin (mg/dL)	4.23	11.53	20.58
aPTT (sec)	41	29	51
PT (%)	60	110	54
INR	1.3	0.9	1.4
AST (U/L)	32	61	63
ALT (U/L)	44	134	65
γGT (U/L)	104	38	205
Alk. phos. (U/L)	327	63	112
Albumin (g/dL)	n.d.	2.35	2.40
COP (mmHg)	23.3	20.7	15.6
Total protein (g/dL)	6.5	6.3	4.5
Main diagnoses	St. p. LTX (FLC), sepsis, pneumonia, ICH, cholangitis liver abscess	St. p. LTX (M. Wilson)	St. p. LTX (FLC), liver abscess, sepsis, pneumonia
Time from LTX	1 month	3 days	8 months
AMB indication	probable pulmonary aspergillosis	pre-emptive therapy of suspected pulmonary aspergillosis	*Candida* cholangitis
Evidence of IFI	microscopic detection of *Aspergillus* in BAL	halo sign at CT, positive serum galactomanan assay	*C. krusei* and *C. glabrata* in bile
MIC of AMB (mg/L)	no positive culture	no positive culture	*C. krusei* 0.25/0.38 *C. glabrata* 0.38
AMB formulation	LAMB	LAMB	ABCD
Dose (mg/d)	250	200	400 (1st dose: 200)
Dose (mg/kg/d)	2.78	3.77	5.56 (1st dose: 2.38)
Day of AMB	1, 7	5	1, 4

Legend to Table 1

CVVH, continuous veno-venous hemofiltration because of acute renal failure; Creatinine, plasma creatinine, normal range 0.70 - 1.20 mg/dL; Bilirubin, total plasma bilirubin, normal range 0.00 - 1.28 mg/dL; aPPT, activated partial thromboplastin time, normal range 26 – 37 sec; PT, prothrombin time, normal range 70 - 130 %; INR, international normalized ratio; AST, aspartate-aminotransferase, normal range 10 - 50 U/L; ALT, alanine-aminotransferase, normal range 10 - 50 U/L; γGT, gamma glutamyl transferase, normal range 10 - 71 U/L; Alk. phos., alkaline phosphatase, normal range 40 – 130 U/L; Albumin, plasma albumin concentration, normal range 4.19 – 5.35; plasma albumin concentration was determined up to 14 days before the study day; Total protein, normal range 6.60 – 8.70; COP, colloid osmotic pressure, normal range 19.0 – 30.0 mmHg; St. p. LTX, status post liver transplantation; FLC, fatty liver cirrhosis; M. Wilson, Wilson disease (hepatolenticular degeneration); ICH, intracranial haemorrhage;; ERCP, endoscopic retrograde cholangiopancreatography; n. a. not applicable; IFI, invasive fungal infection; BAL, broncho-alveolar lavage; CT, computerised tomography; *C., Candida*; AMB, amphotericin B; LAMB, liposomal amphotericin B (AmBisome®); ABCD, amphotericin B colloidal dispersion (Amphocil®, Amphotec®); n. d., not determined

Table 2: Plasma pharmacokinetics of liberated, lipid-bound, and total AMB during treatment with liposomal AMB (LAMB) or AMB colloidal dispersion (ABCD)

	Patient 1						Patient 2			Patient 3					
AMB therapy	LAMB						LAMB			ABCD					
	Single dose			Multiple dose			Multiple doses			Single dose			Multiple doses		
Dose (mg/d)	250			250			200			200			400		
Dose (mg/kg/d)	2.78			2.78			3.77			2.38			5.56		
Day of AMB	1			7			5			1			5		
	LI	LB	**TO**	LI	LB	**TO**	LI	LB	**TO**	LI	LB	**TO**	LI	LB	**TOT**
C_{max} (mg/L)	2.45	4.82	**7.25**	5.15	9.46	**14.60**	2.54	6.99	**9.53**	0.27	0.51	**0.78**	1.10	0.34	**1.45**
C_{min} (mg/L)	1.18	0.34	**1.52**	3.01	1.06	**4.07**	1.59	1.13	**2.72**	0.02	0.01	**0.03**	0.03	0.02	**0.05**
AUC_{0-n}	37.53[a]	28.41[a]	**66.51**[a]	93.19[a]	87.91[a]	**181.63**[a]	33.18[b]	58.34[b]	**106.04**[b]	1.65[a]	1.69[a]	**3.39**[a]	14.09[a]	3.00[a]	**16.13**[a]
$t_{1/2}$ (h)	13.26	6.20	**8.29**	24.58	7.15	**12.01**	26.93	3.93	**6.40**	10.50	9.91	**10.04**	2.84	5.85	**3.34**
CL (ml/h/kg)	50	90	**30**	10	30	**10**	40	60	**30**	1,420	1,520	**730**	390	1,730	**340**
V_d (L/kg)	0.89	0.79	**0.40**	0.51	0.29	**0.20**	1.62	0.33	**0.31**	21.48	21.80	**10.55**	1.60	14.55	**1.63**

Legend to Table 2

AMB, Amphotericin B; LAMB, liposomal AMB; ABCD, AMB colloidal dispersion; LI, liberated AMB; LB, lipid-bound AMB; TO, total AMB; total AMB plasma concentrations were calculated by addition of liberated and lipid-bound concentrations. Thus, a time-total-AMB-concentration curve was obtained. The plasma pharmacokinetics of total AMB was calculated from this curve; the first ABCD dose amounted to 50 percent of the maintenance dose in order to avoid infusion-related toxicity; C_{max}, AMB peak concentration; C_{min} AMB minimum concentration; AUC_{0-n}, area under the AMB concentration-time curve over the sampling period, from 0 (before AMB infusion) to n (time after start of AMB infusion) [a] n = 24 h or [b] n = 16 h; $t_{1/2}$, AMB half-life; CL, AMB clearance; V_d, volume of distribution.

Table 3: *In-vitro* simulation of *Candida* growth in porcine bile and in RPMI culture medium

C. albicans

	no AMB	0.025 mg/L AMB	0.05 mg/L AMB	0.5 mg/L AMB	1.0 mg/L AMB	5.0 mg/L AMB
porcine bile	**++**	**++**	**++**	**++**	**++**	**+/-**
RPMI pH 7.2	**++**	**++**	**++**	**+**	**+/-**	**--**
RPMI pH 7.8	**++**	**++**	**++**	**+**	**+**	**--**

C. glabrata

	no AMB	0.025 mg/L AMB	0.05 mg/L AMB	0.5 mg/L AMB	1.0 mg/L AMB	5.0 mg/L AMB
porcine bile	**+**	**+**	**+**	**+**	**+/-**	**+/-**
RPMI pH 7.2	**++**	**++**	**++**	**++**	**+/-**	**--**
RPMI pH 7.8	**++**	**++**	**++**	**++**	**--**	**--**

C. krusei

	no AMB	0.025 mg/L AMB	0.05 mg/L AMB	0.5 mg/L AMB	1.0 mg/L AMB	5.0 mg/L AMB
porcine bile	**+/-**	**+/-**	**+/-**	**+/-**	**+/-**	**+/-**
RPMI pH 7.2	**++**	**++**	**++**	**++**	**++**	**--**
RPMI pH 7.8	**++**	**++**	**++**	**++**	**+/-**	**--**

C. tropicalis

	no AMB	0.025 mg/L AMB	0.05 mg/L AMB	0.5 mg/L AMB	1.0 mg/L AMB	5.0 mg/L AMB
porcine bile	**++**	**+**	**+**	**+**	**++**	**+/-**
RPMI pH 7.2	**++**	**+**	**++**	**+**	**-**	**--**
RPMI pH 7.8	**++**	**++**	**++**	**+**	**+/-**	**--**

Legend to Table 3:

Candida strains were incubated in amphotericin B-spiked porcine bile and RPMI medium, respectively, for up to 48 h (see 2.3 in the text). Amphotericin B deoxycholate was added at concentrations of 0.0 (control), 0.025, 0.05, 0.5, 1.0 and 5.0 mg/L. *C.*, *Candida*; AMB, amphotericin B deoxycholate; RPMI, RPMI media at pH 7.2 and at pH 7.8 (adjusted to biliary pH); CFU, colony-forming units; - = 10-100-fold decrease in CFU; -- = decrease in CFU by a factor of 100 or more; +/- = neither fungal growth nor fungal decease; + = 10-100-fold increase in CFU; ++ = increase in CFU by a factor of 100 or more;

Table 4: *Ex-vivo* simulation of *candida* growth in patient bile samples

Time from infusion (h)	**0 (before)**	**4**	**6**	**10**	**16**	**24**
Patient 1						
Day 1 of LAMB						
C. tropicalis	+/-	no sample	no sample	+/-	+/-	+/-
AMB (mg/L)	<LLOQ	-	-	0.23	0.16	0.42
Day 7 of LAMB						
C. albicans	no sample	+/-	+/-	+/-	+/-	+/-
AMB (mg/L)	-	0.92	1.21	1.02	1.28	0.88
Patient 2						
Day 5 of LAMB						
C. albicans	no sample	+	+	+	+/-	no sample
AMB (mg/L)	-	0.09	0.40	0.33	0.02	-
Patient 3						
Day 1 of ABCD						
C. krusei	no sample	+	+/-	+/-	+/-	+/-
C. glabrata	no sample	++	+/-	+/-	+/-	+/-
AMB (mg/L)	-	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Day 4 of ABCD						
C. krusei	+	+	+	+	++	+/-
C. glabrata	+/-	+/-	+/-	+/-	+	+/-
AMB (mg/L)	0.02	0.03	0.02	0.03	0.04	0.02

Legend to Table 4:

Patient bile samples retrieved during treatment with liposomal amphotericin B or amphotericin B colloidal dispersion were incubated with *Candida* (10^4 conidia) for a maximum of 48 h (see 2.3 in the text).

Time from infusion, time after start of infusion of LAMB and ABCD, respectively, the infusion time amounted to 4 h; LAMB, liposomal amphotericin B (AmBisome®); ABCD, amphotericin B colloidal dispersion (Amphocil®, Amphotec®); AMB, amphotericin B; +/- = neither fungal growth nor fungal eradication; + = 10-100-fold increase in CFU; ++ = increase in CFU by a factor of 100 or more; LLOQ, lower limit of quantification (< 0.025 mg/L); *C.*, *Candida*